Acta Tropica 126 (2013) 146–149

Contents lists available at SciVerse ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Infectra®-kit: A device for restraining mice and confining tsetse flies during trypanosome infection transmission experiments

Kariuki Ndung'u*, James Karuku Kibugu, Purity Kaari Gitonga, John Kibuthu Thuita, Joanna Eseri Auma, Samuel Kariuki Gitonga, Geoffrey Njuguna Ngae, Grace Adira Murilla

Kenya Agricultural Research Institute (KARI), Kenya

ARTICLE INFO

Article history.
Received 28 November 2012
Received in revised form 28 January 2013
Accepted 3 February 2013
Available online xxx

Keywords:
Infectra®-kit
Mice
Fly vectors
Transmission experiments

ABSTRACT

Chemical (anaesthesia) and manual techniques are commonly used to restrain mice during vector-mediated parasite transmission experiments in the laboratory. Chemical restraint may interfere with natural fly vector–mouse interactions and therefore potentially affect the outcome of transmission experiments. Conversely, manual restraint is labour-intensive and exposes laboratory animals to excessive restraining-related discomfort. We report development of a mouse restraining device (Infectra®-kit) that allows essential transmission studies to be carried out with minimal human manipulation and without the need for anaesthesia. Infectra®-kit can be used as a single unit for restraining one mouse or as eight-assembled units, thus significantly improving efficiency of a single operator in comparison to manual restraint. The kit was validated by comparing feeding success in tsetse flies fed on mice restrained using Infectra®-kit (Group I) to those manually restrained (Group II). The mean ± SE % feeding success was 75.0 ± 8.2% and 82.1 ± 8.2% for tsetse flies in Groups I and II respectively. Statistical analysis using two sample *t*-test showed no significant difference between the two groups at $p \leq 0.05$, indicating that Infectra®-kit as a restraining device was as good as the conventional manual restraint method. The main benefits of using Infectra®-kit for transmission studies therefore include reduction of man-hours and animal restraining-related discomfort. In addition, the risk of accidental injury to laboratory personnel by either mice or tsetse flies is minimized, which is an important consideration when working with zoonotic parasites.

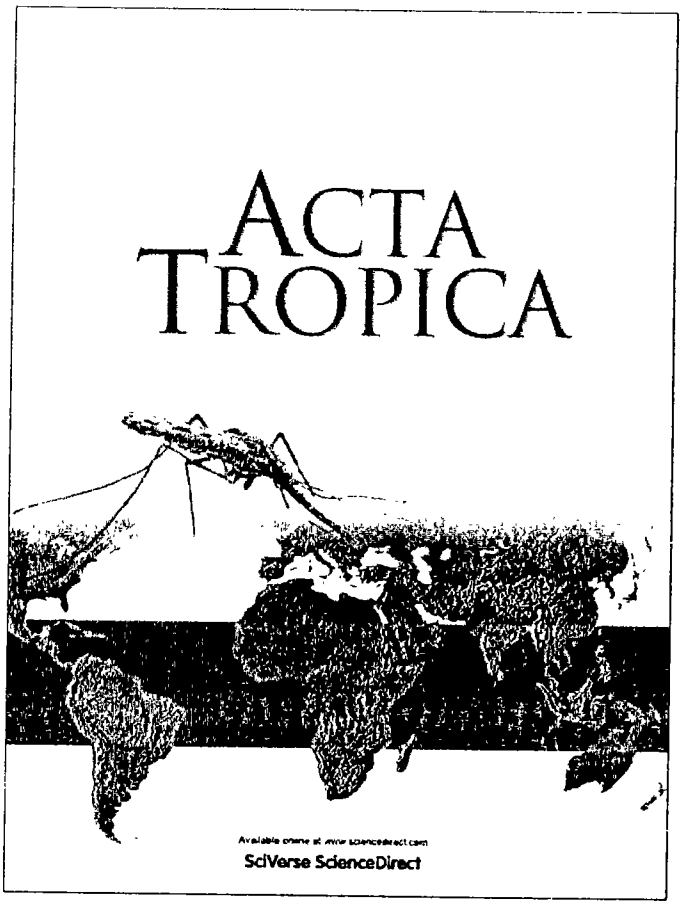
1. Introduction

To study the mechanisms involved in disease transmission, laboratory experiments should be designed in such a way as to mimic natural events as much as possible. Vector parasite transmission approach is therefore probably superior to the traditional needle inoculation method, which eliminates the natural transmission features (Secundino et al., 2012). Laboratory mice are used in studies to elucidate parasite transmission mechanisms in vector-borne diseases such as tsetse fly-transmitted human African trypanosomiasis (HAT). One approach of infecting tsetse flies in the laboratory is by allowing them to feed on trypanosome-infected blood through an artificial membrane (Evans, 1979). Alternatively, tsetse flies can be infected by feeding them on trypanosome-infected donor animals (Okoth et al., 2006). After parasite developmental cycle in the fly vector host, the infected fly is then used for cyclical transmission in un-infected mice (Okoth et al., 2006; Gibson and Bailey, 2003).

Rodent-restraining devices described earlier (Harry, 2001; Toshio and Kanagawa, 2003; Hughes, 1994; Charles and Douglas, 1993; Kazutoshi, 1990) are not designed for twin function of restraining the animal host and also confining the fly vectors. The conventional mouse-restraining (restraining using hands) method for trypanosome-tsetse-mouse transmission studies is labour-intensive. In addition, (1) some flies feed for long periods of time (Mitchell et al., 1976) necessitating prolonged restraining of the mouse, thereby exposing the animals to restraint-related exhaustion; (2) laboratory personnel risk physical injury (bites) and accidental infection with zoonotic parasites from animals and/or fly vectors. Chemical restraint techniques such as animal anaesthesia are not suitable for transmission studies since they may negate natural interaction between the animal host and the fly vectors during transmission experiments. Inhalants are likely to immobilize or kill fly vectors and require a fume hood which is not conducive for these studies. Also, chemical contaminants in blood diets are likely to cause mortality in laboratory flies (Kibugu et al., 2010; IAEA, 2000; Feldmann, 1994). Injectable anaesthetic drugs may have repellant effects, negating host-fly interaction. Furthermore, anaesthesia affects cardiovascular and thermoregulatory

* Corresponding author at: P.O. Box 362, Kikuyu, Kenya. Tel.: +254 720407210.
E-mail address: kariukindungu36@yahoo.com (K. Ndung'u)

0001-706X/$ – see front matter © 2013 Elsevier B.V. All rights reserved
http://dx.doi.org/10.1016/j.actatropica.2013.02.006



*This is a sample cover image for this issue. The actual cover is not yet available at this time.

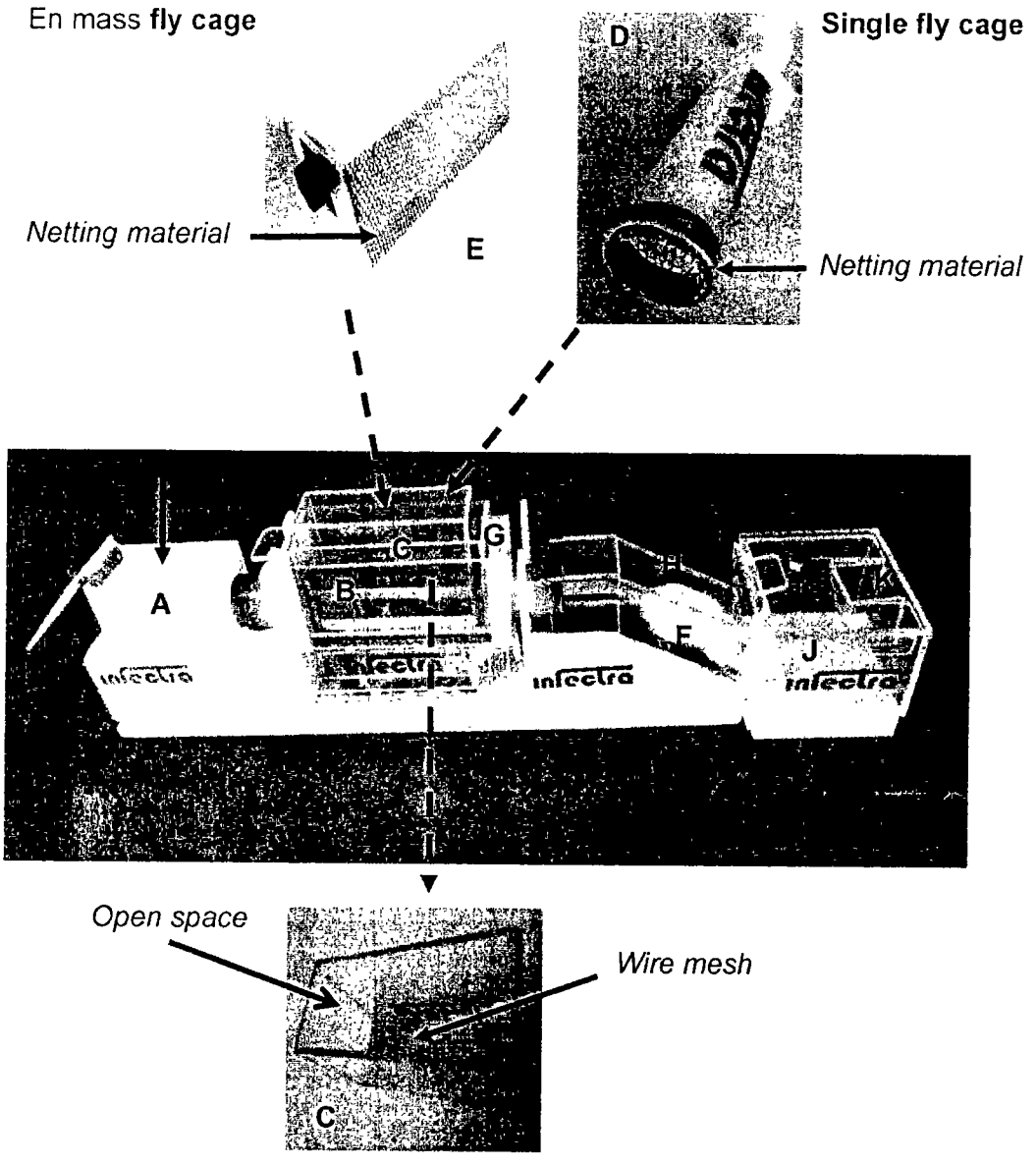


Fig. 1. Photographic presentation of the main components of Infectra®-kit. The mouse is introduced through a cage (A) connected to a tunnel through a door leading to a mouse-holding compartment (B) where it is held using an adjustable restrainer (C). The mouse is exposed to fly vectors through a galvanized wire mesh fitted on the lower side of restrainer as indicated by the arrow. Tsetse flies confined singly in a single-fly cage (D) or as a group in *en mass* fly cage (E) fitted with netting material are introduced by inserting the fly cages through the open hollow space in the restrainer as shown by the arrow and allowed to feed on the mouse. The restrainer is designed to fasten the vector cage in upright position during tsetse-mouse interaction. After the interaction, the mouse (F) is released through an escape door (G) connected to a tunnel and moves down a ladder (H) to a resting cage (J) to which a mouse feeding tray (K) is fitted.

mechanisms (Wheler et al., 2010) which could alter parasite inocula and heat stimulus that attracts fly vectors to the host. To counter these challenges, we developed a mouse- and user-friendly device (Infectra®-kit) suitable for restraining mice and confining tsetse flies.

2. Materials and methods

2.1. *Infectra®-kit*

Fig. 1 shows photographic representation of Infectra®-kit. The main components of the kit are frame with a firm stand and cage (Fig. 1A) for introducing the mouse into a transparent holding compartment (Fig. 1B). A restrainer (Fig. 1C) with adjustable screws, comfortably minimizes animal movement and injury. Experiments with individual fly vectors are conducted using single-fly holding cages (Fig. 1D) that are fitted with pistons for easy fly tractability while *en masse* fly-holding cages (Fig. 1E) are suitable for studies involving many flies. Interaction of mouse and flies is allowed through galvanized wire mesh fitted on the restrainer. The latter is rotated along an axis to facilitate dorsal or ventral exposure of mouse. The mouse (Fig. 1F) is released through an escape door (Fig. 1G) leading to a ladder (Fig. 1H) which facilitates the mouse's movement to a resting cage (Fig. 1J) where it is briefly kept and fed through a trough (Fig. 1K) fitted on top of the cage. Construction material of Infectra®-kit is acrylic. The device can be used as a

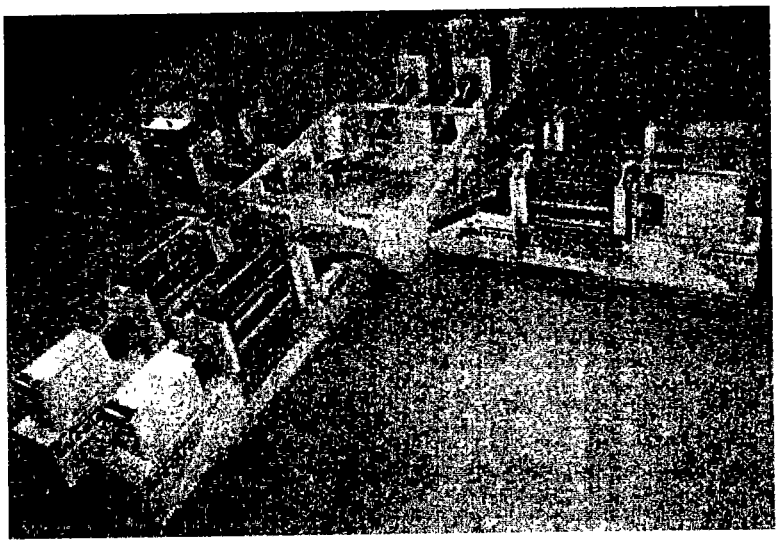
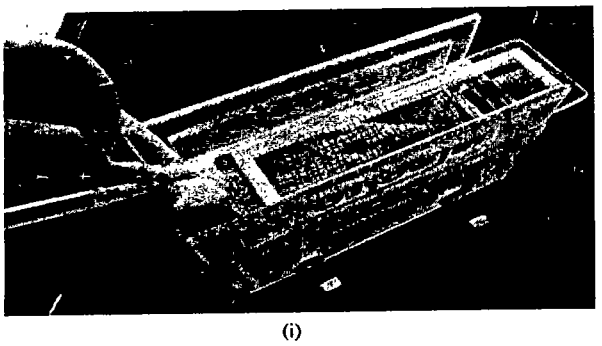


Fig. 2. Assembled Infectra®-kit units. Eight units of the kit can be manned by one operator during trypanosome transmission studies.

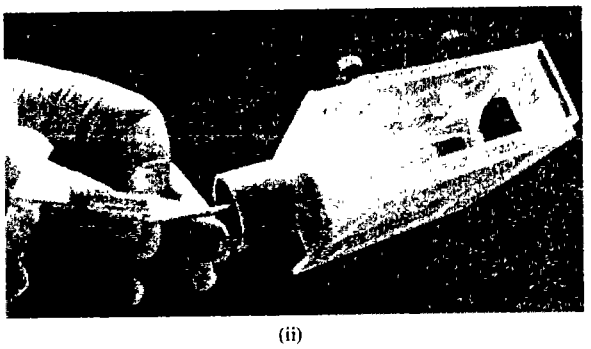
single-unit or eight-assembled units (Fig. 2). The holding compartment is detachable and can be used to restrain the mouse during other veterinary procedures such as blood sample collection (tail snip), intra-peritoneal and intravenous injections (Fig. 3).

2.2. Laboratory mice

Sixteen adult Swiss White mice weighing 30–35 g were received from Kenya Agricultural Research Institute-Trypanosomiasis Research Centre (KARI-TRC) small animal breeding unit and transferred to acclimatization room where they were acclimatized for 1 week before commencement of the experiment. They were maintained on commercial feed (Mice pellets®, Unga Ltd., Kenya), water provided *ad libitum* and wood-chippings used as bedding material.



(i)



(ii)

Fig. 3. Mice restrained in ventral position in the detachable compartment B for administration of intra-peritoneal (i) and intravenous (ii) injections.

2.3. Tsetse flies

One hundred and twelve male *Glossina pallidipes* teneral flies housed in *en masse* cages each holding seven flies were received from KARI-TRC insectaries.

2.4. Ethics

Mice handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of KARI-TRC. Clinical status of the mice was evaluated as outlined by Hoff (2000) before exposure to tsetse flies and found to be normal. Allowing for anaemic mice, the tsetse feeding regime described in this paper is optimal without risk of animal exsanguination (Loder, 1997; Murdoch University, 2010; Amole et al., 1982). After the study, the experimental mice were humanely euthanized and incinerated, and the tsetse flies were killed using chloroform.

2.5. Experimental design and validation of Infectra®-kit

A completely randomized design was used in this study. The flies were divided into two groups (Groups I and II). Group I flies ($n = 7$) were introduced to an Infectra®-kit restrained mouse and allowed to feed for 10 min. Group II flies ($n = 7$) were similarly fed on a hand-restrained (convectional manual restraining method) mouse as for Group I flies. These experiments were replicated eight times and the fly feeding success determined. Engorged fly abdomen was considered indicative of successful feeding as previously reported (Okoth et al., 2006). After exposure to tsetse flies, the clinical status of the mice restrained employing the two restraining techniques was monitored as outlined earlier by Hoff (2000) and compared.

2.6. Data analysis

Feeding success rates of Groups I and II flies were compared employing two sample *t*-test procedure on Genstat statistical package (Genstat 5 Release 3.2 Lawes Agricultural Trust, IACR-Rothamsted) to test significance of difference in performance

between the Infectra®-kit and convectional manual restraining methods respectively.

3. Results

The means ± standard errors of tsetse feeding success was 75.0 ± 8.2% with 95% confidence interval of 58.9–91.1% for Group I flies while for Group II flies, it was 82.1 ± 8.2% with 95% confidence interval of 66.0–98.2%. Using two sample *t*-test assuming equal variances, there was no evidence of significant ($p \leq 0.05$) difference between the two restraining techniques. Infectra®-kit restrained mice were clinically normal with no changes in temperament compared to manually restrained mice which were relatively more docile.

4. Discussion

Vector-mediated parasite transmission studies have historically proved critical to the understanding of certain aspects of infectious disease processes. The link between the tsetse fly and HAT was confirmed between 1895 and 1903 by Dr. Gordon Bruce who conclusively demonstrated that the flies transmit the disease from sick to healthy hosts (WHO, 2012). In the laboratory, vector parasite transmission studies are applied in xenodiagnosis of *Trypanosoma cruzi*, babesiosis and HAT; xenodiagnosis is frequently more reliable in diagnosis of low parasitaemia than microscopy (Masiga et al., 1992; Thuita et al., 2008). Furthermore, parasite transmission experiments are useful in elucidation of the underlying mechanisms of parasites, invertebrate and vertebrate hosts, as well as the complex interactions between the three, which support disease onset and persistence (Secundino et al., 2012). In this communication, we report development of a device for restraining mice and confining fly vectors that facilitates such essential transmission studies.

The validation results showed that feeding success for tsetse flies fed on mice restrained using Infectra®-kit (Group I) was equal to those that were fed on mice restrained using the gold standard method *i.e.* manual hand restraint (Group II). Indeed the level of feeding success observed in the present study agrees with data of previous experiments in our laboratory (Dr. Thuita, unpublished data). Experience in our laboratory and elsewhere (Van et al., 2010) indicates that infected tsetse flies take relatively longer time than uninfected flies to feed which necessitates longer man-hours during transmission studies. A key advantage of Infectra®-kit is that several units can be assembled together, thus reducing the number of mice handlers and man-hours required for transmission experiments. In addition, the kit would facilitate standardization of mouse-restraining procedures which improves consistency of results generated in transmission experiments. The kit is portable and can be used in the field during disease surveillance studies with parasites that grow in mice. Although chemical restraining techniques are sometimes used during transmission studies (Secundino et al., 2012; Caljon et al., 2006), they have limitations since chemicals can induce fly mortality (Kibugu et al., 2010; IAEA, 2000; Feldmann, 1994) and alter animal physiology (Wheler et al., 2010) negating host-fly interaction. Where it is desirable to conduct transmission studies under field-like conditions, use of this device will allow natural interaction of the fly vector and mice, and enhance the welfare of the laboratory animal.

We recommend Infectra®-kit as a replacement of the convectional manual-restraining technique for trypanosome-tsetse-mice transmission studies, blood sampling, as well as intra-peritoneal and intravenous injections. However it should be further validated prior for use in studies involving other vector-borne diseases. This technology is a modification of a device previously patented under Kenya Patent No. KE/P/4/00409 by the first author (Ndung'u, 2006).

Acknowledgements

We thank the Director KARI and Dr. Mathu Ndung'u (former Director, Kenya Trypanosomiasis Research Institute) for financial support, Richard Kurgat, Daniel Nyaga, Phylis Alusi and laboratory staff (KARI-TRC) for technical expertise, Dr. Karanja Kibicho, David Chitai (Jomo Kenyatta University of Agriculture and Technology) and Mr. Kiranga (Jua kali artisan) for fabricating the invention. Stanley Atsali and Paul Chege (Kenya Industrial Property Institute) who took this invention through the whole patenting process.

References

Amole, B.O., Clarkson, A.B., Shear, H.L., 1982. Pathogenesis of anaemia in *Trypanosoma brucei*-infected mice. Infect. Immun. 36 (3), 1060–1068.
Caljon, G., Van, D.A.J., Stijlemans, B., Coosemans, M., De, B.P., Magez, S., 2006. Tsetse fly saliva accelerates the onset of *Trypanosoma brucei* infection in a mouse model associated with a reduced host inflammatory response. Infect. Immun. 74 (11), 6324–6330.
Charles, W.C., Douglas, M., 1993. Kolodny H. Method and apparatus for mass producing insects entomopathogens and entomoparasites. US patent, 5,178,094. http://www.google.com/patents/US5178094
Evans, D.A., 1979. Cyclical transmission of *Trypanosoma brucei rhodesiense* and *Trypanosoma congolense* by tsetse flies infected with culture-form procyclic trypanosomes. J. Protozool. 26, 425–427
Feldmann, U., 1994. Some quality control parameters used in rearing of tsetse flies. In: Ochieng'-Odero, J.R.P. (Ed.), Techniques of Insect Rearing for Development of Integrated Pest and Vector Management Strategies, vol. 1. Proceedings of an International Group Training Course on Development of Integrated Pest and Vector Management Strategies. 16 March–3 April 1992, icipe, Nairobi, Kenya. icipe, Science Press, pp. 15–29.
Gibson, W., Bailey, M., 2003. The development of *Trypanosoma brucei* within the tsetse fly midgut observed using green fluorescent trypanosomes. Kinetoplastid Biol. Dis. 2 (1), 1.
Harry, E.K., 2001. Apparatus and method for trapping and monitoring mice and insects. US patent, 6: 202,339B1. http://www.google.com/patents/US6202339
Hoff, J., 2000. Methods of blood collection in the mouse. Lab. Anim. 29 (10), 47–53.
Hughes, P.R., 1994. High density rearing system for larvae. US patent 1994; 5,351,643. http://www.google.com/patents/US5351643
IAEA, 2000. Development of cost-effective diets for use in mass production of tsetse flies. In: Report and recommendations of a Consultants' Group Meeting organized by the Joint FAO/IAEA Division of Nuclear Applications Food and Agriculture, Vienna, Austria, 17–21 July 2000.
Kazutoshi, T., 1990. Insect keeping and observing container. US patent 4,924,810. http://www.patentgenius.com/patent/4924810.html
Kibugu, J.K., Kiragu, J.M., Mumba, A.M., Mwangangi, D.M., Muchiri, M.W., 2010. Improved protocol for aseptic collection and handling procedures of bovine blood diet in areas with special contamination challenges for use in tsetse rearing. Lab. Anim. 44, 281–282.
Loder, P.M.J., 1997. Size of blood meals taken by tsetse flies (*Glossina spp.*) (Diptera: Glossinidae) correlates with fat reserves. Bull. Entomol. Res. 87, 547–549.
Masiga, D.K., Smyth, A.J., Ayes, P., Bromidge, T.J., Gibson, W.C., 1992. Sensitive detection of trypanosomes in tsetse flies by DNA amplification. Int. J. Parasitol. 22, 909–918.
Mitchell, B.K., Henriette, A., Reinouts Van, H., 1976. A comparison of the feeding behavior in teneral and post-teneral *Glossina morsitans* (Diptera, Glossinidae) using an artificial membrane. Entomol. Exp. Appl. 20 (2), 105–112.
Murdoch University, 2010. Blood collection recommendations for Murdoch University Research and Teaching Projects. See www.research.murdoch.edu.au/ethics/arec/BloodSop.rtf (accessed 18.03.10).
Ndung'u, K., 2006. Vector and rodent holding devices during parasite transmission experiments in the laboratory. www.wipo.int/patentscope/search/en/WO2006121222
Okoth, S.O., Kokwaro, E.D., Kiragu, J.M., Murilla, G., 2006. Susceptibility and transmissibility of capacity off subpopulations of *Glossina pallidipes* to Human infective *Trypanosomes brucei rhodesiense*. Trends Med. Res. 1, 75–85.
Secundino, N.F.C., Freitas, V.C., Monteiro, C.C., Pires, A.A.M., David, B.A., Pimenta, P.F.P., 2012. The transmission of *Leishmania infantum chagasi* by the bite of the *Lutzomyia longipalpis* to two different vertebrates. Parasit. Vectors 5, 20.
Toshio, W., Kanagawa, J.P., 2003. Milking apparatus for laboratory animals. US patent 2003/0051668 A1. http://www.patents.com/us-20030051668.html
Thuita, J.K., Kagira, J.M., Mwangangi, D., Matovu, E., Turner, C.M., Masiga, D., 2008. Trypanosoma brucei rhodesiense transmitted by a single tsetse fly bite in vervet monkeys as a model of human African trypanosomiasis. PLoS Negl. Trop. Dis. 2, e238.
Van, D.A.J., Caljon, G., De, R.K., De, B.P., Coosemans, M., 2010. *Trypanosoma brucei* modifies the tsetse salivary composition, altering the fly feeding behavior that favors parasite transmission. PLoS Pathog. 6 (6), e1000926.
Wheler, C., Nelles, P., Schueller, N., Hudy, C., 2010. Rodent anesthesia. In: Rodent Anesthesia Wetlab 26th Annual Conference and Trade Show Saskatchewan Association of Veterinary Technologists Inc., November 5–7, Saskatoon.
World Health Organisation, 2012. The History of Sleeping Sickness. http://www.who.int/trypanosomiasis_african/country/history/en/index3.html